



Sodium hypochlorite: A promising agent for reducing *Botrytis cinerea* infection on rose flowers

Andrew J. Macnish^a, Kristy L. Morris^a, Annemarie de Theije^a, Manon G.J. Mensink^c, Henry A.M. Boerrigter^c, Michael S. Reid^a, Cai-Zhong Jiang^{b,*}, Ernst J. Woltering^{c,**}

^a Department of Plant Sciences, University of California, One Shields Avenue, Davis, CA 95616, USA

^b Crops Pathology and Genetics Research Unit, USDA-ARS, One Shields Avenue, Davis, CA 95616, USA

^c Agrotechnology & Food Sciences Group, Wageningen University and Research Centre, PO Box 17, 6700 AA Wageningen, The Netherlands

ARTICLE INFO

Article history:

Received 13 March 2010

Accepted 30 July 2010

Keywords:

Botrytis cinerea

Flowers

Sodium hypochlorite

Postharvest

Rosa × hybrida

ABSTRACT

Botrytis cinerea is a fungal pathogen that greatly reduces the postharvest quality of rose flowers. A postharvest dip in 200 $\mu\text{L L}^{-1}$ sodium hypochlorite (NaOCl) for 10 s at 20 °C provided the greatest control of *B. cinerea* on 'Akito' and 'Gold Strike' flowers. NaOCl derived from Clorox® Ultra household bleach solution was more effective than laboratory grade NaOCl in reducing disease symptoms. Lowering the pH of the NaOCl solution from pH 9.7 (unadjusted) to pH 7.0 greatly improved its efficacy. Treating 'Gold Strike' flowers in this pH-adjusted NaOCl solution was more effective in reducing the level of infection on petals than postharvest dips in the conventional fungicides Medallion®, Phyton®, Switch® and Vanguard®. Applying NaOCl prior to a 3- or 10-d commercial shipment also provided the most consistent disease control for a wide range of rose cultivars as compared to conventional fungicides. Of particular interest, the efficacy of NaOCl and Phyton® was greatest when these compounds were applied to 'Gold Strike' flowers after incubation at 20 °C and 90% RH for 6–9 h. Our findings highlight NaOCl as a promising new candidate for the control of *B. cinerea* on rose flowers.

Published by Elsevier B.V.

1. Introduction

Botrytis cinerea Pers. is a ubiquitous fungal pathogen that causes gray mold in many fruit, vegetable and ornamental crops (Schwinn, 1992). Rose, gerbera and chrysanthemum are among affected cut flower species (Elad, 1988). Infection by *B. cinerea* reduces the postharvest quality of rose flowers leading to substantial economic loss by growers and wholesalers (Vrind, 2005). The infection process in rose begins with deposition of conidia on petals during flower development (Kerssies et al., 1995). Disease symptoms are usually first visualized on petals after harvest as small quiescent lesions or pocks (Pie and De Leeuw, 1991). When exposed to humid (>93% relative humidity; RH) conditions, such as during transport and storage, lesions become necrotic and can spread to infect entire petals (Salinas et al., 1989; Williamson et al., 1995).

Synthetic chemical fungicides (e.g. benzimidazoles, dicarboximides) have long been relied on to reduce *B. cinerea* disease development (Elad and Shtienberg, 1995). However, continued use of these fungicides is problematical owing to development of fun-

gal resistance and increasing social and environmental concerns over chemical residues (Williamson et al., 2007). Several alternative strategies for reducing *B. cinerea* infection on rose flowers have been tested including exposure to elevated CO₂ atmospheres (Hammer et al., 1990), inoculation with antagonistic microbes (Elad et al., 1993; Tatagiba et al., 1998) and treatment with calcium sulfate (De Capdeville et al., 2005). However, none of these treatments consistently provided complete control of this pathogen.

Sodium hypochlorite (NaOCl), a chlorine-containing compound, is a strong oxidizer with broad spectrum antimicrobial activity (Bloomfield, 1996). When dissolved in water, NaOCl ionizes to Na⁺ and the hypochlorite ion in equilibrium with hypochlorous acid (HOCl), the active moiety (Dychdala, 1983). HOCl damages microbe cell membranes, proteins and nucleic acids by oxidative degradation upon contact (McDonnell and Russell, 1999). NaOCl is a relatively inexpensive and non-residual chemical that is widely used to reduce bacterial and fungal contamination on fruit and vegetable surfaces, processing equipment, and in flower vase solutions (Suslow, 1997; van Doorn et al., 1990). Commonly used NaOCl concentrations vary from 50 to 200 mg L⁻¹ depending on the commodity and mode of application. Aside from one brief study by Elad (1988) whereby treatment with the high concentration of 1% NaOCl for 2 min did not prevent *B. cinerea* infection on rose petals, there are no reports on the potential for this oxidizer to reduce disease on cut flowers.

* Corresponding author. Tel.: +1 530 752 7060; fax: +1 530 752 4554.

** Corresponding author. Tel.: +31 317 480 116; fax: +31 317 483 011.

E-mail addresses: cjiang@ucdavis.edu (C.-Z. Jiang), Ernst.woltering@wur.nl (E.J. Woltering).

In the present study, we hypothesized that postharvest treatment of rose flowers with low NaOCl concentrations would reduce the development of *B. cinerea* on petals. We determined the optimal NaOCl concentration, treatment time and pH of the solution. The activity of NaOCl relative to conventional fungicides and its efficacy to reduce disease on rose flowers subjected to commercial shipment were also evaluated.

2. Materials and methods

2.1. Biological material

Rose (*Rosa × hybrida*) flowers were harvested at commercial maturity (i.e. outer petals starting to reflex) from farms near Watsonville, CA, USA, and Quito, Ecuador. Cultivars with a known high susceptibility to natural infection with *B. cinerea* were selected based on observations by commercial growers. Flowers from California were transported dry to the laboratory in Davis, CA, in an air-conditioned (20 °C) car within 3 h unless otherwise stated. Flowers from Ecuador were shipped dry to our laboratory in 10 d in accordance with commercial practice. A *Botrytis* QuickStix™ kit (model AS 049 OR; EnviroLogix™, Portland, ME, USA) which utilizes a monoclonal antibody to detect *B. cinerea* fungus (Meyer and Dewey, 2000) was used to confirm flower infection. Briefly, 0.1 g of petal tissue was sampled from flowers at the end of each experiment and ground in the kit extraction buffer in accordance with the manufacturer. A test strip was then placed into the tissue suspension for 10 min. Development of a visible band on the strip indicated the presence of *B. cinerea*.

2.2. Optimal NaOCl concentration

Cut 'Akito' and 'Gold Strike' stems were randomly assigned to treatments and re-cut to 50 cm. All leaves that would otherwise be submerged in vase solution were detached, leaving the uppermost three leaves intact. Entire flower heads were dipped to a depth of 10 cm in deionized water containing 0, 50, 100, 200, 400 or 800 $\mu\text{L L}^{-1}$ NaOCl (provided as Clorox® Ultra, a.i. 6.15% NaOCl; The Clorox Co., Oakland, CA, USA) for 10 s at 20 °C. Stems were then placed individually into plastic vases containing a commercial vase solution (10 g L^{-1} Chrysal Clear Professional 3; Chrysal International B.V., Naarden, The Netherlands). A low density polyethylene bag was placed over each stem to create 90% RH around flowers to encourage maximum development of natural *B. cinerea* infection. Stems in bags were maintained for evaluation for 7 d at 20 °C under cool white fluorescent bulbs that provided 18 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of light (12 h d^{-1}) at flower height. Flowers were assessed daily for the presence of *B. cinerea* infection. Disease incidence was expressed as the proportion (%) of infected flowers in a treatment. Flower quality was evaluated during the 7-d study as described by Macnish et al. (2010).

2.3. Optimal NaOCl treatment time

The optimal duration of the NaOCl dip treatment was determined by immersing 'Akito' and 'Gold Strike' flower heads in deionized water containing 0 or 200 $\mu\text{L L}^{-1}$ NaOCl (provided as Clorox® Ultra; The Clorox Co.) for 0, 1, 5, 10 or 30 s at 20 °C. Stems were then placed into vases and maintained for evaluation as described above.

2.4. Efficacy of different preparations of NaOCl

Aliquots of laboratory grade NaOCl (5.65–6%, Fisher Scientific Inc., Fair Lawn, NJ, USA), or the commercial bleach products Clorox® Ultra, Hi-lex® Bleach (a.i. 5.25–5.50% NaOCl; Hi-lex Corp., Egan,

MN, USA) and Sierra Bleach (a.i. 5.25% NaOCl; Sierra Chemical Co., Stockton, CA, USA) were diluted in deionized water to give 200 $\mu\text{L L}^{-1}$ NaOCl for treatment. 'Gold Strike' flowers were dipped in the NaOCl solutions for 10 s as previously described. Additional control stems were either dipped in deionized water (0 $\mu\text{L L}^{-1}$ NaOCl) or not dipped in solution. Flowers were then maintained for evaluation.

2.5. Optimal pH of NaOCl dip solution

Since the availability of the active HOCl increases with a decrease in NaOCl solution pH (Dychdala, 1983), we established a study to determine the efficacy of NaOCl treatments of different pH to reduce *B. cinerea* infection. A 200 $\mu\text{L L}^{-1}$ NaOCl (provided as Clorox® Ultra) solution (pH 9.7) was prepared and adjusted to pH 5, 6, 7, 8 or 9 by adding aliquots of 1 N HCl (ACS grade, Fisher Scientific Inc.). 'Gold Strike' flowers were dipped in the NaOCl solutions for 10 s at 20 °C as described above. Additional flowers were not dipped in solution and served as controls. Flowers were then transferred to vases for evaluation.

2.6. Activity of NaOCl solution over time

Deionized water containing 0 or 200 $\mu\text{L L}^{-1}$ NaOCl (provided as Clorox® Ultra; pH 7) was prepared in duplicate sets and maintained inside the laboratory at 20 °C and 18 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of light. 'Gold Strike' flowers were dipped in the first set of solutions for 10 s as previously described. After 8 h, additional flowers were dipped in the second set of solutions. In a separate experiment, different subsets of flowers were repeatedly dipped for 10 s in a 200 $\mu\text{L L}^{-1}$ NaOCl solution over 8 h at 20 °C. Following treatment, all flowers were maintained for evaluation as described above.

2.7. Activity of NaOCl relative to conventional fungicides

'Gold Strike' flowers were dipped for 10 s in deionized water containing 200 $\mu\text{L L}^{-1}$ NaOCl (provided as Clorox® Ultra; pH 7) or commercial fungicides Medallion® 50 WP (0.6 g L^{-1} , a.i. 50% fludioxonil; Syngenta Crop Protection Inc., Greensboro, NC, USA), Phyton® 27 (1 mL L^{-1} , a.i. copper sulfate; Source Technology Biologicals, Inc., Edina, MN, USA), Switch® 62.5 WG (0.6 g L^{-1} , a.i. 37.5% cyprodinil, 25% fludioxonil; Syngenta Crop Protection Inc.) and Vanguard® 75 WG (0.6 g L^{-1} , a.i. 75% cyprodinil; Syngenta Crop Protection Inc.). Additional control stems were either dipped in deionized water (0 $\mu\text{L L}^{-1}$ NaOCl; pH 7) or not dipped in solution. Flowers were then placed into vases for evaluation.

2.8. Efficacy of NaOCl treatment prior to short- or long-term commercial shipping

For short-term shipping, 'Akito', 'Blue Bird', 'Gold Strike', 'Kardinal' and 'Orlando' flowers were harvested from the California farm, combined into 25-stem bunches and cut to 50 cm in accordance with commercial practice. Flowers were dipped in deionized water containing 0 or 200 $\mu\text{L L}^{-1}$ NaOCl (provided as Clorox® Ultra; pH 7) or Phyton® 27 (1 mL L^{-1} ; Source Technology Biologicals) for 10 s as described above. Additional control flowers were not dipped in solution. Flower bunches were placed into a commercial hydration solution (2 mL L^{-1} Chrysal Clear Professional 1; Chrysal International B.V.) for 24 h at 2 °C. They were then packed into fiberboard flower boxes and transported by refrigerated truck from the farm to the Davis laboratory via a wholesaler in Sacramento, California, in 3 d. For the long-term shipping, 'Blushing Akito', 'Cherry Brandy', 'Engagement', 'Mohana', 'Polar Star' and 'Topaz' flowers were harvested from the farm in Ecuador. Flowers were immediately dipped in water containing 0 or 200 $\mu\text{L L}^{-1}$ NaOCl (provided

as Clorox® Ultra; pH 7) or the commercial fungicides Polar® 10 WP (0.25 mL L⁻¹, a.i. 10% polyoxin B; Sumitomo Corp., Ecuador), Switch® 62.5 WG (0.6 g L⁻¹; Syngenta Crop Protection Inc.), or Teldor® Combi 416.7 SC (1.5 mL L⁻¹, a.i. 350 g L⁻¹ fenhexamid, 66.7 g L⁻¹ tebuconazole; Bayer CropScience) for 10 s as previously described. Stems were then prepared for export as described above. They were transported in flower boxes by airplane and refrigerated truck from the farm to the laboratory via Miami, Florida in 10 d. At the laboratory, stems from both shipments were re-cut and maintained for evaluation as described above.

2.9. Optimal timing of NaOCl treatment

The optimal time to apply NaOCl treatment to flowers after harvest was determined. 'Akito' and 'Gold Strike' flowers were harvested from the California farm and placed immediately into a commercial hydration solution (2 mL L⁻¹, Chrysal Clear Professional 1; Chrysal International B.V.) at 20 °C. Flowers were enclosed within a low density polyethylene bag that served to increase the RH therein. They were removed from the bag after 0, 3, 6 and 9 h incubation and dipped in deionized water containing 0 or 200 µL L⁻¹ NaOCl (provided as Clorox® Ultra; pH 7) or the commercial fungicide Phyton® 27 (1 mL L⁻¹; Source Technology Biologicals) for 10 s as described above. Stems were then maintained in vases for evaluation as described above.

2.10. Experiment design and data analysis

Data are presented from a series of experiments that each included common treatments to provide replication over time. Experiments were repeated once and representative data are shown. Depending upon the experiment, 20–25 replicate flowers were assigned to each treatment. Flowers in vases were arranged in a completely randomized block design for evaluation. Disease incidence data were presented as binary responses; either infected (1) or not infected (0). Pairwise comparisons between treatments were completed using Fisher's exact test (two-sided) with the MULTTEST procedure of SAS (Version 9.1; SAS Institute Inc., Cary, NC). The Bonferroni adjustment was used to control the overall Type I error rate for each experiment (Miller, 1981).

3. Results

3.1. Optimal NaOCl concentration

A 10 s postharvest dip in 100 µL L⁻¹ NaOCl solution greatly reduced the incidence of *B. cinerea* infection on 'Gold Strike' petals (Fig. 1). Increasing the NaOCl concentration to 200 and 400 µL L⁻¹ also maintained low levels of disease (Fig. 1) and had no adverse affect on flower quality during the 7 d of this study (data not shown). Treatment with 800 µL L⁻¹ NaOCl prevented disease development but was associated with slight petal bleaching (data not shown). Similar responses to NaOCl treatments were also observed for 'Akito' flowers (Fig. 1).

3.2. Optimal NaOCl treatment time

Immersing flowers into 200 µL L⁻¹ NaOCl for just 1 s was sufficient to reduce the development of *B. cinerea* on 'Gold Strike' (Fig. 2) and 'Akito' (data not shown) petals. Extending the NaOCl treatment time to 5, 10 and 30 s prevented disease.

3.3. Efficacy of different preparations of NaOCl

Treating 'Gold Strike' flowers for 10 s in four different proprietary 200 µL L⁻¹ NaOCl solutions markedly reduced infection on

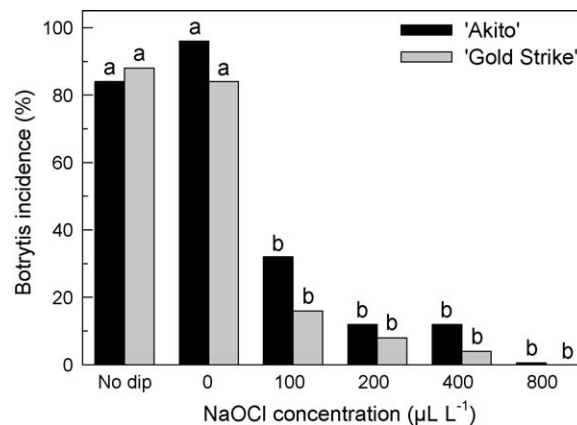


Fig. 1. The incidence of *Botrytis cinerea* infection on *Rosa × hybrida* 'Akito' and 'Gold Strike' flowers after a 7-d vase life at 20 °C and 90% RH. Flowers were dipped in 0, 100, 200, 400 or 800 µL L⁻¹ NaOCl (provided by Clorox® Ultra bleach solution) for 10 s at 20 °C on day 0 of vase life. Additional control flowers were not dipped in solution. Within each cultivar, data (n = 25) followed by different letters are significantly different ($P \leq 0.05$).

petals (Fig. 3). Dipping flowers in NaOCl provided by Clorox® Ultra prevented disease symptoms. Treatment with NaOCl derived from Hi-lex® bleach, Sierra bleach and laboratory grade NaOCl resulted in 5, 15 and 25% of flowers developing *B. cinerea*, respectively.

3.4. Optimal pH of NaOCl solution

Lowering the pH of the 200 µL L⁻¹ NaOCl solution from pH 9.7 (unadjusted) to pH 5.0–8.0 greatly improved its efficacy as a 10-s dip treatment to reduce infection on 'Gold Strike' flowers (Fig. 4). Treatment with NaOCl solution of pH 7.0 prevented disease development.

3.5. Activity of NaOCl solution over time

Dipping flowers in freshly prepared or 8-h old 200 µL L⁻¹ NaOCl solutions of pH 7 were equally effective in reducing the incidence of disease on 'Gold Strike' petals (data not shown). Similarly, repeated dipping of different subsets of flowers in 200 µL L⁻¹ NaOCl over 8 h did not significantly reduce the activity of the treatment despite an accumulation of organic matter in solution (data not shown).

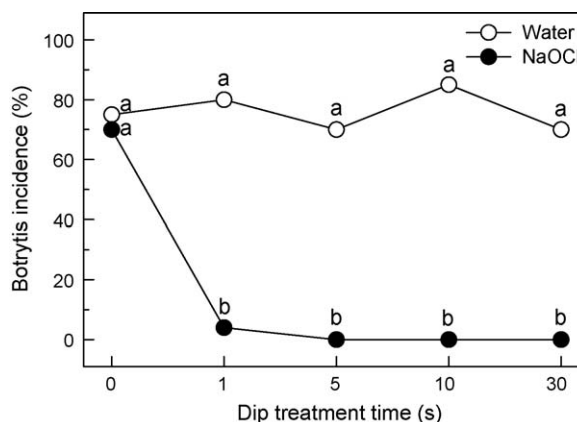


Fig. 2. The incidence of *Botrytis cinerea* infection on *Rosa × hybrida* 'Gold Strike' flowers after a 7-d vase life at 20 °C and 90% RH. Flowers were dipped in 0 (water) or 200 µL L⁻¹ NaOCl (provided by Clorox® Ultra bleach solution) for 1, 5, 10 or 30 s at 20 °C on day 0 of vase life. Data (n = 20) followed by different letters are significantly different ($P \leq 0.05$).

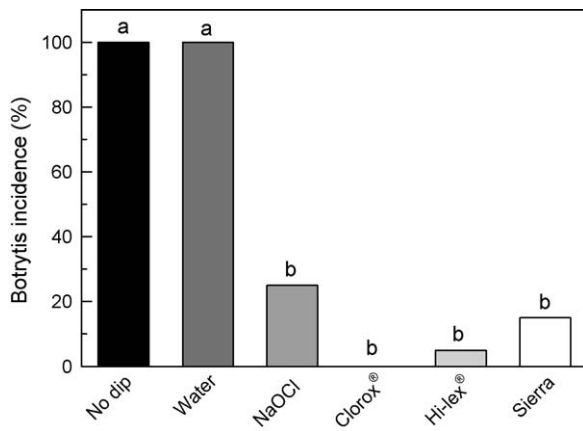


Fig. 3. The incidence of *Botrytis cinerea* infection on *Rosa × hybrida* 'Gold Strike' flowers after a 7-d vase life at 20 °C and 90% RH. Flowers were dipped in 0 (water) or 200 $\mu\text{L L}^{-1}$ NaOCl provided by laboratory grade NaOCl, or Clorox® Ultra, Hi-lex® and Sierra commercial bleach solutions for 10 s at 20 °C on day 0 of vase life. Additional control flowers were not dipped in solution. Data ($n=20$) followed by different letters are significantly different ($P \leq 0.05$).

3.6. Activity of NaOCl relative to conventional fungicides

Treating 'Gold Strike' flowers in 200 $\mu\text{L L}^{-1}$ NaOCl of pH 7 for 10 s was more effective in reducing the level of infection on petals than postharvest dips in the conventional fungicides Medallion®, Phytan®, Switch® and Vangard® (Fig. 5). Of the fungicides, Switch® and Phytan® showed the greatest potential to reduce *B. cinerea* development on petals.

3.7. Efficacy of NaOCl treatment for commercial shipments

Treating flowers in 200 $\mu\text{L L}^{-1}$ NaOCl at pH 7 or 1 mL L^{-1} Phytan® 27 for 10 s prior to a 3-d commercial shipment markedly reduced the subsequent development of *B. cinerea* on 'Akito', 'Blue Bird', 'Gold Strike', 'Kardinal' and 'Orlando' petals (Fig. 6). Treatment with 200 $\mu\text{L L}^{-1}$ NaOCl for 10 s before a 10-d intercontinental shipment also reduced infection on 'Blushing Akito', 'Cherry Brandy', 'Engagement', 'Polar Star' and 'Topaz' flowers (Table 1). Relative to treatment with Polar®, Switch® and Teldor® Combi fungicides, which were highly variable in activity, NaOCl provided the most consistent reduction in disease development on petals of all six

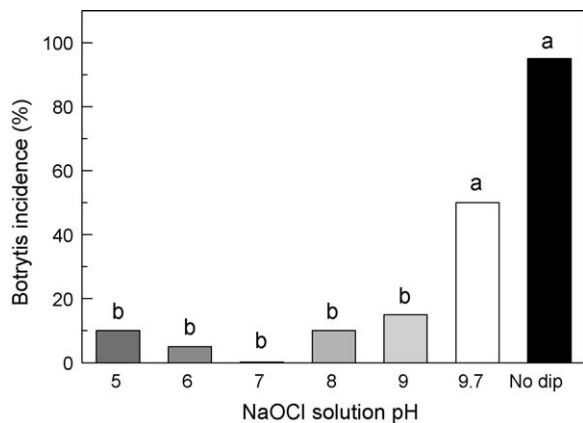


Fig. 4. The incidence of *Botrytis cinerea* infection on *Rosa × hybrida* 'Gold Strike' flowers after a 7-d vase life at 20 °C and 90% RH. Flowers were dipped in 200 $\mu\text{L L}^{-1}$ NaOCl (provided by Clorox® Ultra bleach solution) adjusted to pH 5, 6, 7, 8, 9 or 9.7 (unadjusted) for 10 s at 20 °C on day 0 of vase life. Additional control flowers were not dipped in solution. Data ($n=20$) followed by different letters are significantly different ($P \leq 0.05$).

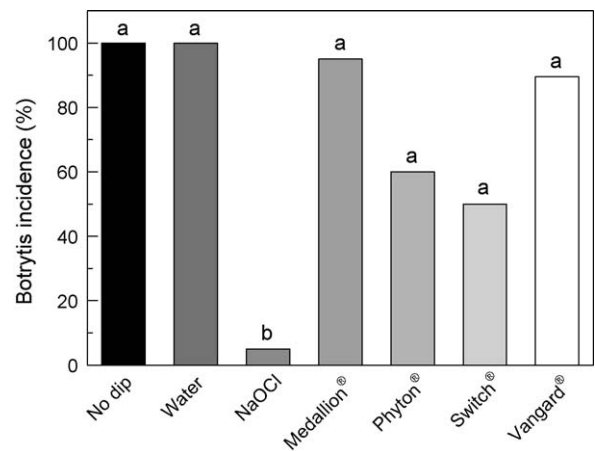


Fig. 5. The incidence of *Botrytis cinerea* infection on *Rosa × hybrida* 'Gold Strike' flowers after a 7-d vase life at 20 °C and 90% RH. Flowers were dipped in 0 (water) or 200 $\mu\text{L L}^{-1}$ NaOCl (provided by Clorox® Ultra bleach solution; pH 7), or 0.6 g L^{-1} Medallion®, 1 mL L^{-1} Phytan®, 0.6 g L^{-1} Switch®, and 0.6 g L^{-1} Vangard® fungicide solutions for 10 s at 20 °C on day 0 of vase life. Additional control flowers were not dipped in solution. Data ($n=20$) followed by different letters are significantly different ($P \leq 0.05$).

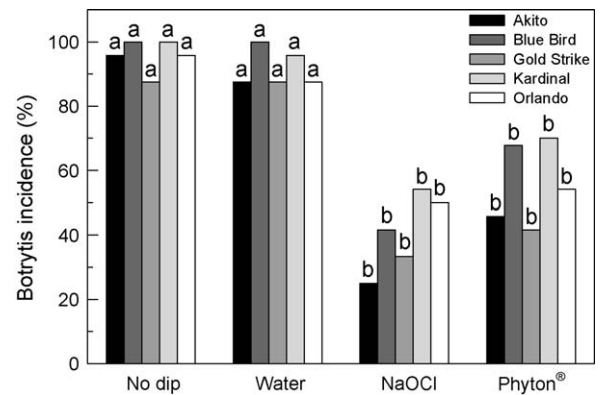


Fig. 6. The incidence of *Botrytis cinerea* infection on *Rosa × hybrida* 'Akito', 'Blue Bird', 'Gold Strike', 'Kardinal' and 'Orlando' flowers after a 7-d vase life at 20 °C and 90% RH. Flowers were harvested and dipped in 0 (water) or 200 $\mu\text{L L}^{-1}$ NaOCl (provided by Clorox® Ultra commercial bleach solution; pH 7), or 1 mL L^{-1} Phytan® fungicide for 10 s at 20 °C. Additional control flowers were not dipped in solution. Flower stems were placed into a hydration solution for 24 h at 2 °C and then transported dry to the laboratory in 3 d. Data ($n=24$) followed by different letters are significantly different ($P \leq 0.05$).

cultivars (Table 1). While all cultivars were susceptible to *B. cinerea* infection, 'Mohana' had less disease development than the other cultivars.

3.8. Optimal timing of NaOCl treatment

The efficacy of NaOCl and Phytan® 27 treatments was greatest when these compounds were applied to 'Gold Strike' flowers after a 6- and 9-h incubation at 20 °C and 90% RH (Fig. 7). The 6-h incubation was also associated with a substantial reduction in disease on control stems that were subsequently dipped in water. A similar treatment response (better control when flowers were pre-incubated for 6 h) was observed for 'Akito' flowers (data not shown).

4. Discussion

A postharvest dip in 200 $\mu\text{L L}^{-1}$ NaOCl for 1–10 s at 20 °C represents a simple, safe and reliable treatment for reducing the

Table 1

The incidence of *Botrytis cinerea* infection on *Rosa × hybrida* 'Blushing Akito', 'Cherry Brandy', 'Engagement', 'Mohana', 'Polar Star' and 'Topaz' flowers after a 7-d vase life at 20 °C and 90% RH. Flowers were harvested and dipped in 0 (water) or 200 $\mu\text{L L}^{-1}$ NaOCl provided by Clorox® Ultra commercial bleach solution (pH 7), or 0.25 mL L^{-1} Polar®, 0.6 g L^{-1} Switch®, or 1.5 mL L^{-1} Teldor® Combi fungicide for 10 s at 20 °C. Flower stems were placed into a hydration solution for 24 h at 2 °C and then transported dry to the laboratory in 10 d. Data ($n=20$) for each cultivar followed by different letters are significantly different ($P \leq 0.05$). 'NS' denotes no significant difference ($P > 0.05$).

Cultivar	Fungicide dip treatment				
	Water	NaOCl	Polar®	Switch®	Teldor® Combi
'Blushing Akito'	90 a	50 b	50 b	80 a	0 c
'Cherry Brandy'	80 a	20 b	0 b	60 a	80 a
'Engagement'	90 a	40 b	50 b	100 a	90 a
'Mohana' ^{NS}	40	20	40	60	50
'Polar Star'	70 a	30 b	80 a	90 a	30 b
'Topaz'	80 a	40 b	0 c	40 b	0 c

incidence of *B. cinerea* infection on rose flowers (Figs. 1 and 2). Our findings highlight the rapid antifungal activity of this oxidizer and are consistent with reports that immersing pear and tomato fruit into NaOCl solution can also reduce surface infection by fungal pathogens such as *B. cinerea* (Spotts and Peters, 1982; Bartz et al., 2001). We also confirmed that neutralizing the NaOCl treatment solution from pH 9.7 to pH 7 improved its efficacy to further reduce disease on flowers (Fig. 3). The disinfecting efficiency of NaOCl is reported to increase with a decrease in pH in association with greater availability of HOCl (Dychdala, 1983). While treatment with acidic NaOCl solutions also maintained low disease levels on flowers (Fig. 3), these solutions liberate chlorine gas, a human respiratory irritant, and would be unsuitable for commercial use (Luttrell, 2001).

NaOCl treatment of rose flowers was more effective in reducing *B. cinerea* infection than several registered fungicides when tested under laboratory conditions (Fig. 5). Applying NaOCl prior to commercial shipment also provided the most consistent disease control for a broad range of rose cultivars (Fig. 6, Table 1). While the relatively poor and variable efficacy of the fungicides was unexpected, it may reflect resistant populations of *B. cinerea* (Williamson et al., 2007). Similarly, sole reliance on chlorination can also lead to development of NaOCl-resistant microbes (Ridgway and Olson, 1982; McDonnell and Russell, 1999). Accordingly, careful attention to maintaining an integrated disease control strategy that includes NaOCl is recommended. Considerable variation in the susceptibility to infection also existed among the different cultivars as previously reported by Hammer and Evensen (1994). We also noted variance

in efficacy of the NaOCl treatment; 200 $\mu\text{L L}^{-1}$ was normally effective, but in the pH experiment, this concentration was only effective when the pH was adjusted to near neutral. In this experiment, the disease pressure was particularly high (controls showed near 100% infection), which may explain the disappointing results from un-neutralized NaOCl. This genotypic variation and potential differences in the natural infection levels on flowers highlights the difficulty in consistently controlling this pathogen by one strategy alone (Kerssies et al., 1995).

We found that household bleach solutions containing NaOCl were effective for use as a postharvest dip treatment (Fig. 4). From a commercial perspective, these products are relatively inexpensive and readily available, and could easily be incorporated into postharvest disease control practices. NaOCl is already approved for contact sterilization of fruit and vegetables (Food and Drug Administration, 2009). Among the proprietary bleach products, Clorox® Ultra provided the greatest control of *B. cinerea* on flowers, and surprisingly was a more effective disinfectant than laboratory grade NaOCl. Presumably, the composition of inactive ingredients (NaCl, surfactants, colorants) in this commercial formulation accounts for the observed differences in treatment efficacy.

An 8-h-old NaOCl solution was just as effective as a freshly prepared dip treatment when maintained at 20 °C under low levels of artificial light. We also found that the NaOCl solution remained effective against *B. cinerea* when it was used repeatedly to treat flowers over 8 h. We observed moderate accumulation of organic matter in the NaOCl solution but this did not diminish the solution activity. Previous studies have established that the concentration of available chlorine in NaOCl solutions and its associated antimicrobial activity is rapidly reduced following the addition of organic matter such as that on cut flower stem surfaces (Dychdala, 1983; Xie et al., 2008). Chlorine in NaOCl solutions is also highly sensitive to degradation by ultra-violet radiation, and care should be taken to complete postharvest dip treatments away from direct sunlight (Bloomfield, 1996).

Exposure of *B. cinerea* conidia to free water or high RH can stimulate germ tube formation in just 6–8 h at 20 °C (Hawker and Hendy, 1968; Pie and De Leeuw, 1991). Our observation that maintaining flowers at 20 °C and 90% RH for 6–9 h before applying NaOCl and Phyton® 27 fungicide enhanced treatment efficacy (Fig. 7) suggests that germinating conidia are more susceptible to biocides than dormant spores (McDonnell and Russell, 1999). We also showed that dipping flowers in water after 6 h at 90% RH reduced disease development (Fig. 7). Previous work by Williamson et al. (1995) established that *B. cinerea* spores incubated at high RH produced shorter and more loosely attached germ tubes than conidia exposed to free water. Thus, it is possible that dipping flowers in water after high RH incubation physically removed some spores. That germinating spores are potentially more vulnerable to postharvest treatments provides an interesting opportunity to further study and improve disease control on roses.

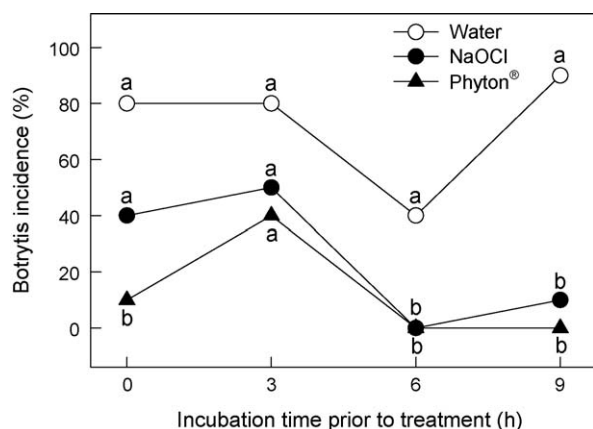


Fig. 7. The incidence of *Botrytis cinerea* infection on *Rosa × hybrida* 'Gold Strike' flowers after a 7-d vase life at 20 °C and 90% RH. Flowers were harvested and maintained at 20 °C and 90% RH for 0, 3, 6 and 9 h prior to dipping in 0 (water) or 200 $\mu\text{L L}^{-1}$ NaOCl (provided by Clorox® Ultra commercial bleach; pH 7), or 1 mL L^{-1} Phyton® fungicide for 10 s at 20 °C on day 0 of vase life. Data ($n=20$) followed by different letters are significantly different ($P \leq 0.05$).

Acknowledgements

We thank the Maersk Line, the Joseph H. Hill Memorial Foundation, and the USDA Floriculture Initiative for partial funding of this study. We also acknowledge Sireena Chieng for technical assistance with experiments, and Jerome Braun, Angadh Singh, Yin Jin and Hui Wang for advice on statistics.

References

- Bartz, J.A., Eayre, C.G., Mahovic, M.J., Concelmo, D.E., Brecht, J.K., Sargent, S.A., 2001. Chlorine concentration and the inoculation of tomato fruit in packinghouse dump tanks. *Plant Dis.* 85, 885–889.
- Bloomfield, S.F., 1996. Chlorine and iodine formulations. In: Ascenzi, J.M. (Ed.), *Handbook of Disinfectants and Antiseptics*. Marcel Dekker, Inc., New York, NY, USA, pp. 133–158.
- De Capdeville, G., Maffia, L.A., Finger, F.L., Batista, U.G., 2005. Pre-harvest calcium sulfate applications affect vase life and severity of gray mold in cut roses. *Sci. Hort.* 103, 329–338.
- Dychdala, G.R., 1983. Chlorine and chlorine compounds. In: Block, S.S. (Ed.), *Disinfection, Sterilization, and Preservation*. Lea and Febiger, Philadelphia, PA, USA, pp. 157–182.
- Elad, Y., 1988. Latent infection of *Botrytis cinerea* in rose flowers and combined chemical and physiological control of the disease. *Crop Protect.* 7, 361–366.
- Elad, Y., Kirshner, B., Gotlib, Y., 1993. Attempts to control *Botrytis cinerea* on roses by pre- and postharvest treatments with biological and chemical agents. *Crop Protect.* 12, 69–73.
- Elad, Y., Shtienberg, D., 1995. *Botrytis cinerea* in greenhouse vegetables: chemical, cultural, physiological and biological controls and their integration. *Integr. Pest Manage. Rev.* 1, 15–29.
- Food and Drug Administration, 2009. Indirect Food Additives: Adjuvants, Production Aids, and Sanitizers, Code of Federal Regulations Title 21, Volume 3, Part 178, Section 178.1010 <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=178.1010>.
- Hammer, P.E., Evensen, K.B., 1994. Differences between rose cultivars in susceptibility to infection by *Botrytis cinerea*. *Phytopathology* 84, 1305–1312.
- Hammer, P.E., Yang, S.F., Reid, M.S., Marois, J.J., 1990. Postharvest control of *Botrytis cinerea* infections on cut roses using fungistatic storage atmospheres. *J. Am. Soc. Hort. Sci.* 115, 102–107.
- Hawker, L.E., Hendy, R.J., 1968. An electron-microscope study of germination of conidia of *Botrytis cinerea*. *J. Gen. Microbiol.* 33, 43–46.
- Kerssies, A., Bosker-van Zessen, A.I., Frinking, H.D., 1995. Influence of environmental conditions in a glasshouse on conidia of *Botrytis cinerea* and on post-harvest infection of rose flowers. *Eur. J. Plant Pathol.* 101, 201–216.
- Luttrell, W., 2001. Toxic tips: sodium hypochlorite. *Chemical Health and Safety* 8, 24–26.
- Macnish, A.J., Leonard, R.T., Borda, A.M., Nell, T.A., 2010. Genotypic variation in the postharvest performance and ethylene sensitivity of cut rose flowers. *HortScience* 45, 790–796.
- McDonnell, G., Russell, A.D., 1999. Antiseptics and disinfectants: activity, action and resistance. *Clin. Microbiol. Rev.* 12, 147–179.
- Meyer, U.M., Dewey, F.M., 2000. Efficacy of different immunogens for raising monoclonal antibodies to *Botrytis cinerea*. *Mycol. Res.* 104, 979–987.
- Miller, R.G., 1981. *Simultaneous Statistical Inference*, 2nd ed. Springer Verlag, New York.
- Pie, K., De Leeuw, G.T.N., 1991. Histopathology of the initial stages of the interaction between rose flowers and *Botrytis cinerea*. *Netherlands J. Plant Pathol.* 97, 335–344.
- Ridgway, H.F., Olson, B.H., 1982. Chlorine resistance patterns of bacteria from two drinking water distribution systems. *Appl. Environ. Microbiol.* 44, 972–987.
- Salinas, J., Glandorf, D.C.M., Picavet, F.D., Verhoeff, K., 1989. Effects of temperature, relative humidity and age of conidia on the incidence of spotting on gerbera flowers caused by *Botrytis cinerea*. *Netherlands J. Plant Pathol.* 95, 51–64.
- Schwinn, F.J., 1992. Significance of fungal pathogens in crop production. *Pest. Outlook* 3, 18–28.
- Spotts, R.A., Peters, B.B., 1982. Use of surfactants with chlorine to improve pear decay control. *Plant Dis.* 66, 725–727.
- Suslow, T., 1997. *Postharvest Chlorination, Basic Properties and Key Points for Effective Disinfection*. Publication 8003. University of California, Oakland, CA, USA.
- Tatagiba, J., da, S., Maffia, L.A., Barreto, R.W., Alfenas, A.C., Sutton, J.C., 1998. Biological control of *Botrytis cinerea* in residues and flowers of rose (*Rosa hybrida*). *Phytoparasitica* 26, 1–12.
- van Doorn, W.G., de Witte, Y., Perik, R.R.J., 1990. Effect of antimicrobial compounds on the number of bacteria in stems of cut rose flowers. *J. Appl. Bacteriol.* 68, 117–122.
- Vrind, T.A., 2005. The *Botrytis* problem in figures. *Acta Hort.* 669, 99–102.
- Williamson, B., Duncan, G.H., Harrison, J.G., Harding, L.A., Elad, Y., Zimand, G., 1995. Effect of humidity on infection of rose petals by dry-inoculated conidia of *Botrytis cinerea*. *Mycol. Res.* 99, 1303–1310.
- Williamson, B., Tudzynski, B., Tudzynski, P., van Kan, J.A.L., 2007. *Botrytis cinerea*: the cause of grey mould disease. *Mol. Plant Pathol.* 8, 561–580.
- Xie, L., Joyce, D.C., Irving, D.E., Eyre, J.X., 2008. Chlorine demand in cut flower vase solutions. *Postharvest Biol. Technol.* 47, 267–270.